

U.S. PATENT APPLICATION
SERIAL NO.: 09/830,514
AMENDMENT B

ATTY DOCKET: 3968.057

IN THE DRAWINGS

Please replace the figures in pages 52 to 75 with the twenty four attached replacement sheets also enumerated 52 to 75 and which contain amended Figures 2a to 2r.

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REMARKS/ARGUMENTS

Review and reconsideration of the Office Action dated November 3, 2004, is respectfully requested in view of the above amendments and the following remarks.

The pending claims are Claims 17, and 19-31.

Claims 24-28 were withdrawn by the Examiner as being drawn to a non-elected invention.

Claims 17, 19-23, and 29-31 are being examined.

Office Action

Turning to the Office Action, the paragraphing of the Examiner is adopted.

Specification

The Examiner contends that there is no Brief Description of the Drawings section. The Examiner has required proper correction.

Applicants respectfully submit that the Brief Description of the Drawings section was inserted by preliminary amendment submitted on April 27, 2001 by previous attorneys representing applicants (see Preliminary Amendment of April 27, 2001, pages 2 and 3). However, the Brief Description of the Drawings section was inserted in the wrong place of the specification.

In response, Applicants have amended the specification deleting the wrongly submitted Brief Description of the Drawings section, and resubmitting a Brief Description of the Drawings section to be inserted between the Summary of the Invention section and the Detailed Description of the Invention section.

Thus, Applicants respectfully submit that the defect with respect to the Brief Description of the Drawings section has been corrected.

The Examiner has also required correction of a minor typographical error in page 32, line 9 of the specification.

Applicants have amended the specification at page 32, line 9 to correct the typographical error cited by the Examiner.

Therefore, Applicants respectfully request the Examiner to withdraw the objection to the specification.

Drawings

The Examiner indicated that there are no figures corresponding to what is described in page 33 as Figures 2a-2r. The Examiner also required that if the drawings depict

gene sequences, to place proper sequence identifiers in the figures or in the Brief Description of the Drawings section.

In response, Applicants submit herewith twenty four replacement sheets corresponding to pages 52-75 which contain amended Figures 2a-2r adding proper labels.

In addition, Applicants have amended the specification by inserting a Brief Description of the Drawings section with proper sequence identifiers which correspond to the amended Figures 2a-2r.

Applicant respectfully requests the Examiner to approve the amended drawings and the changes in the specification. Accordingly, withdrawal of the objection to the drawings is respectfully requested.

Claim Objections

The Examiner has objected claims 17, 19-20 and 29-31 because the recitation "transformed ... organism", "organism according ...", and process for ...". The Examiner requires adding the proper preceding grammatical article ("A", "An" or "The").

The Examiner has also objected claims 21-23 due to the recitation of "an organism to claim X ..." since the organism has been defined in a previous claim. The Examiner suggests

replacing "an" with "the".

Moreover, the Examiner objected claim 19 because of the recitation "one or more genes encoding the enzymes ... is/are altered". The Examiner suggested replacing "is\are" with "are".

Furthermore, the Examiner objected claim 21 due to the recitation of "group consisting of a microorganism, a plant or animal cell". The Examiner suggests amending the claim to recite "group consisting of a microorganism, a plant cell, or an animal cell".

In response, Applicants, following the Examiner suggestions, submit herewith the following amendments to the claims:

- In each of claims 17, 19-20 and 29-31, the proper preceding grammatical article ("A", "An" or "The") has been added.
- In each of claims 21-23, the term "An" has been replaced with the term "The".
- In claim 19, the term "is/are" has been deleted.
- In claim 21, the term "cell" has been inserted.

Therefore, in view of the submitted amendments to claims 17, 19-23 and 29-31, Applicants respectfully request the

Examiner to withdraw the objections to these claims.

Claims Rejections - 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 29-31 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner alleges that claim 29 is indefinite in the recitation of "preparation of alcohols, aldehydes, and organic acids comprising the step of adding an organism comprising enzymes ... such that the intermediates ... accumulate". The Examiner indicates that claim 29 lacks recited steps as to the claimed process for the production of any alcohol, aldehyde and organic acid. The Examiner has assumed that claim 29 is directed to process for the preparation of coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid. The Examiner requires proper correction.

The Examiner also contends that claim 30 is indefinite in the recitation of "wherein the alteration in eugenol ... metabolism is achieved by microbiological culturing methods". The Examiner notes that it is unclear as to which microbiological culturing methods would allow the skilled artisan to insert Ω elements or deletions in a gene. The Examiner has not given weight to the term. The Examiner has interpreted the claim as being drawn to a method for preparing

the organism of claim 17. The Examiner requires proper correction.

Furthermore, the Examiner indicates that claim 31 is indefinite in the recitation of "process for preparing an organism according to claim 29" since claim 29 is directed to a process for the preparation of alcohols, aldehydes, and organic acids. The Examiner has assumed that the recitation refers to claim 17. The Examiner has required proper correction.

The Examiner notes that claims 30-31 are indefinite for omitting essential steps, such omission amounting to a gap between the steps. The Examiner alleges that claims 30-31 lack steps indicating as to how the organism is made. The Examiner has required proper correction.

In response Applicants have amended claim 29 to delete the term "the step of" and to specify that intermediates of the eugenol and/or ferulic acid catabolism pathway accumulate because of the inactivation of the enzymes of this pathway "by inserting . . . or introducing deletions".

Applicants have also amended claim 30 to indicate that the microbiological culturing methods are used to further propagate the claimed organism. Furthermore, Applicants respectfully submit that microbiological culturing methods are

well known in the art of the invention whereby mutants with gene deletions or extra-gene elements may be further propagated.

Moreover, Applicants have amended claim 31 to refer to claim 17, instead of claim 29.

Applicants respectfully submit that microbiological culturing methods and recombinant DNA methods may be interpreted in the art of the invention as steps toward obtaining the organism of the instant invention. In fact, in Example 6 of the specification, Applicants describe conjugation of mutants leading to the generation of organisms with inactivated enzymes for the eugenol and/or ferulic acid catabolism

Therefore, Applicants respectfully submit that claims 29-31, as amended, are not indefinite under 35 U.S.C. 112, second paragraph. Consequently, Applicants respectfully request the withdrawal of the rejection under 35 U.S.C. 112, second paragraph.

Claim Rejections - 35 U.S.C. §112, First Paragraph

WRITTEN DESCRIPTION

The Examiner has rejected claims 17, 19-23 and 29-31 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Examiner

contends that claims 17, 19-23 and 29-31 contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Specifically, the Examiner alleges that the specification discloses no more than the mutagenesis of *Pseudomonas* sp. HR199 by the insertion of Ω elements in the genes encoding the enzymes involved in eugenol and/or ferulic acid catabolism. The Examiner asserts that the specification fails to describe (1) multicellular organisms modified such that their endogenous eugenol/ferulic acid catabolism genes have been modified by insertion of Ω elements or deletions, (2) the structure of all genes from unicellular or multicellular sources encoding enzymes associated with the eugenol/ferulic acid metabolism which if inactivated would create an accumulation of coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid, and (3) other methods to inactivate any enzyme associated with the eugenol/ferulic acid catabolism, such as addition of inhibitors.

The Examiner indicates that the genus of organisms claimed is extremely large and highly variable in structure. The Examiner notes that the written description of a genus may be achieved by the recitation of a representative number of polynucleotides defined by their nucleotide sequence or

structural features common to all members or a substantial portion of members of the genus. The Examiner indicates that in the instant case there is no representative structural feature to all members of the genus.

The Examiner further contends that the description of a genus by structural homology with the *Pseudomonas* sp. HR199 genes is not satisfactory since the art (the Examiner cites several references) teaches the unpredictability of structural homology. Therefore, the Examiner asserts, in the absence of any additional information correlating structure with the desired enzymatic function, or any correlation between the structures of the *Pseudomonas* sp. HR199 genes disclosed and the desired function, many structurally unrelated genes are encompassed by the genus.

The Examiner alleges that the specification only discloses a single species of the claimed organisms, *Pseudomonas* sp. HR199 mutagenized such that the endogenous genes encoding the enzymes of the eugenol/ferulic acid catabolism are inactivated by the insertion of Ω elements or gene deletion, and one method to obtain inactivation of an enzyme, i.e. Ω element insertion or gene deletion, which is insufficient to put one of ordinary skill in the art in possession of all the attributes and features of the claimed invention. The examiner concludes that the skilled artisan cannot reasonable conclude that the applicant had possession

of the claimed invention at the time the instant application was filed.

In response Applicants have amended claim 17 to emphasize that the organisms claimed are those in which the enzymes of the eugenol and or ferulic acid catabolism have been inactivated by inserting Ω elements or introducing deletions into the genes encoding such enzymes.

Applicants respectfully submit that in the present invention Applicants **are not claiming** a genus or the specific genes coding for enzymes of the eugenol\ferulic acid catabolism.

The invention Applicants are claiming specifically refer to the transformed organisms in which the genes encoding for the enzymes of the eugenol and or ferulic acid catabolism have been inactivated by the insertion of Ω elements or introduction of deletions into the genes encoding such enzymes.

Therefore, Applicants respectfully submit that at the time the application was filed, Applicants had possession of organisms in which the enzymes of the eugenol and or ferulic acid catabolism had been inactivated by the insertion of Ω elements or introduction of deletions into the genes encoding such enzymes.

ENABLEMENT

The Examiner has rejected claims 17, 19-23 and 29-31 under 35 U.S.C. 112, first paragraph, allegedly because the specification, while being enabling for

(1) a transformed *Pseudomonas* sp. HR 199 as described in the specification, wherein said strain contains at least one inactivated gene encoding eugenol/ferulic acid catabolism enzymes, wherein said gene inactivation is due to the introduction of deletions or insertional mutagenesis with Ω elements,

(2) a method of using said *Pseudomonas* strain for the production of coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid, and

(3) a method of making the strain of (1),

does not reasonably provide enablement for

(a) any unicellular or multicellular organism modified such that any enzyme associated with eugenol/ferulic acid catabolism is inactivated by inserting Ω elements or deletions in the genes encoding said enzymes, and wherein the intermediates coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid accumulate,

(b) any unicellular or multicellular organism modified such that any gene encoding eugenol/ferulic acid catabolism enzymes

is inactivated by insertion of Ω elements or deletions,
(c) a method to produce coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid with the organisms of (a) or (b), or
(d) a method of making the organisms of (a) or (b).

Then, according to the Examiner, the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The Examiner enumerates the criteria for undue experimentation to indicate that the scope of the claims as described is not commensurate with the enablement provided in regard to the extremely high number of unknown unicellular and multicellular organisms which can be modified as recited in the claims such that the accumulation of coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid is achieved, as well as the extremely large number of genes of unknown structure which are required for inactivation.

The Examiner alleges that the specification discloses no more than the mutagenesis of *Pseudomonas* sp. HR199 by the insertion of Ω elements in the genes encoding the enzymes involved in eugenol and/or ferulic acid catabolism. However, the Examiner asserts that the specification fails to describe

(1) multicellular organisms modified such that their endogenous eugenol/ferulic acid catabolism genes have been modified by insertion of Ω elements or deletions, (2) the structure of all genes from unicellular or multicellular sources encoding enzymes associated with the eugenol/ferulic acid metabolism which if inactivated would create an accumulation of coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid, and (3) other methods to inactivate any enzyme associated with the eugenol/ferulic acid catabolism, such as addition of inhibitors.

The Examiner notes that the art teaches the unpredictability of structural homology to determine function. According to the Examiner, since the structure determines function, one of skill in the art would require some knowledge or guidance as to which are the structural elements in any gene which are characteristic of genes encoding enzymes associated with eugenol or ferulic acid catabolism. Therefore, the Examiner concludes that due to the lack of relevant examples, the amount of information provided, the lack of knowledge about the structural elements required for genes encoding a enzyme associated with eugenol and/or ferulic acid catabolism, and the unpredictability of the prior art in regard to function based on homology, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to practice the claimed invention. Thus, according to the examiner, Applicant has not provided

sufficient guidance to enable one of ordinary skill in the art to make and use the invention in a manner reasonably correlated with the scope of the claims.

It is noted that if the claims were to be amended to refer specifically to *Pseudomonas* sp. HR199, a biological deposit may be required to satisfy the enablement requirements set forth in 35 U.S.C. 112, first paragraph.

Applicants respectfully emphasize that the novelty of the invention is mutant organisms in which Ω elements have been inserted or deletions have been introduced to inactivate the genes encoding the enzymes of the eugenol\ferulic acid catabolism such that the intermediate products accumulate.

Applicants respectfully submit that the techniques and methods used in the present invention are well known in the art of the invention. The techniques and methods described in the specification to obtain mutant organisms with inactivated genes encoding the enzymes for the eugenol and/or ferulic acid metabolism even if only applied to a strain of *pseudomona*, are well known to work for other organisms. In fact, the insertion of Ω elements into genes for other purposes was known in the art.

The examples presented in the specification are sufficiently enabling for the skilled artisan to obtain

organisms in which the genes encoding the enzymes for the eugenol and/or ferulic acid catabolism pathway have been inactivated, such that intermediates of said catabolism pathway accumulate. Moreover, the specification teaches the inactivation of multiple genes encoding an enzyme of the eugenol\ferulic acid metabolism.

Therefore, the specification satisfy the enablement requirement under 35 U.S.C. § 112, first paragraph.

Consequently, Applicants respectfully request the Examiner to withdraw the rejection of claims 17, 19-23 and 29-31 under 35 U.S.C. 112, first paragraph.

Claim Rejections - 35 U.S.C. § 102

The Examiner has rejected claim 29 under 35 U.S.C. § 102(b) as allegedly anticipated by Priefert et al. (J. Bacteriol.179(8): 2592-2607, 1997; previously cited by the Examiner).

The Examiner contends that claim 29 as interpreted is directed to a process for the production of coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and/or vanillic acid wherein an organism is modified such that enzymes involved in the catabolism of eugenol and/or ferulic acid are inactivated. The Examiner notes that Priefert et al. teaches the chemical mutagenesis of *Pseudomonas* sp. HR199 wherein the

vdh gene, which encodes vanillin dehydrogenase, is inactivated. According to the Examiner, inactivation of vanillin dehydrogenase would allow for the accumulation of vanillin.

Applicants have amended claim 29 to recite "by inserting of Ω elements, or introducing deletions, into corresponding genes,".

Applicants respectfully submit that Priefert et al. does not teach the inactivation of genes in *Pseudomonas* sp HR199 by inserting Ω elements or introducing deletions. A reference anticipates only if discloses all the elements of the invention.

In Priefert et al. a non-targeted chemical mutagenesis of *Pseudomonas* sp. was performed as a general approach. Then, several genes of this strain could have been inactivated in addition to the vdh gene. Priefert et al. does not describe the specific targeted inactivation of the vdh gene.

Since, Priefert et al. does not disclose inserting Ω elements or introducing deletions to specifically inactivating genes encoding the enzymes of the eugenol/ferulic acid catabolism, Priefert et al. does not anticipate claim 29, as amended, under 35 U.S.C. § 102(b).

Therefore, Applicants request the withdrawal of the rejection to claim 29 under 35 U.S.C. § 102(b).

Claim Rejections - 35 U.S.C. §103

The Examiner has rejected claims 17, 19-23 and 30-31 under 35 U.S.C. 103(a) as being unpatentable over Priefert et al. (J. Bacteriol. 179(8): 2592-2607, 1997; previously cited by the Examiner) in view of Blondelet-Rouault et al. (Gene 190:315-317, 1997).

According to the Examiner, it would have been obvious for the skilled artisan at the time the invention was made to use the antibiotic resistance gene cassettes of Blondelet-Rouault et al. for insertional mutagenesis of the vdh gene in the Pseudomonas strain of Priefert et al.. The Examiner asserts that one of ordinary skill in the art has a reasonable expectation of success at using insertional mutagenesis to disrupt the vdh gene in Pseudomonas with the antibiotic resistance gene cassettes of Blondelet-Rouault et al. since Ω elements (Ω interposon) for insertion mutagenesis in bacteria is well known and widely used in the art (the Examiner points at page 315, right column, first paragraph). Therefore, The Examiner concludes, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

Applicants respectfully submit that neither of the two,

Priefert et al. or Blondelet-Rouault et al., suggests or motivates combining them.

Priefert et al. describes the non-specific chemical mutagenesis of *Pseudomonas* sp. in which more than one gene could have been inactivated. Moreover, Priefert et al. teaches mutants which could not utilize vanillin. Priefert et al. does not teach the accumulation of intermediate products in the eugenol\ferulic acid catabolism.

However, Priefert et al does not teach the specific inactivation of the genes encoding coniferyl alcohol dehydrogenases, coniferyl aldehyde dehydrogenases, feruloyl-CoA synthetases, enoyl-CoA hydratase-aldolases, beta-ketothiolases, vanillin dehydrogenases or vanillic acid demethylases by the insertion of Ω -elements and/or the introduction of deletions, such that the intermediate products of the eugenol/ferulic acid catabolism accumulate.

In contrast, the present invention describes the specific inactivation of the genes encoding the enzymes of the eugenol/ferulic acid catabolism such that intermediate products of this pathway accumulate.

Thus, Priefert et al. and Blondelet-Rouault et al. can not make obvious an invention that neither of the two references teaches. Therefore, claims 17, 19-23 and 30-31 under 35 U.S.C. 103(a) are not unpatentable over Priefert et

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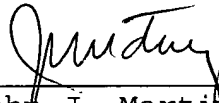
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al. in view of Blondelet-Rouault et al.

Consequently, Applicants respectfully request the withdrawal of the rejection to claims 17, 19-23 and 30-31 under 35 U.S.C. 103(a).

Applicants believe that all the claims are now allowable. Favorable consideration and early issuance of the Notice of Allowance are respectfully requested. Should further issues remain prior to allowance, the Examiner is respectfully requested to contact the undersigned at the indicated telephone number.

Respectfully submitted,



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Enclosures: 24 replacement sheets (Drawings).